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[From the Department of Physiology of Columbia University, New York]

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I. — Introduction

THE use of variations in the concentration of sugar in the blood as an indication of the response of the animal to experimental conditions offers many theoretical advantages over the use of the presence of, or variations in the amount of, sugar in the urine. This is true, first, because changes in either direction may be detected. While sugar is always present in the blood, it is ordinarily present in the urine in minimal quantities only. The urine, therefore, can ordinarily be used to show only an increase in mobilized sugar, while the blood will show either an increase or a decrease. Secondly, profound changes of concentration of sugar may occur in the blood without giving rise to a detectable glycosuria. This may be due to the short duration of the change, or the change may not be of sufficient magnitude to lead to the excretion of sugar by the kidneys or it may possibly be due to a modification of the kidneys themselves. This very sensitiveness may, however, lead to serious difficulties in the handling of the animals before and during any experiment which involves the estimation of sugar in the blood. The third and most fundamental advantage lies in the intimate relation which exists, on the one hand, between the blood and the cells which are using the sugar and, on the other, between the blood and the stores of carbohydrate.

Presumably it is the greater difficulty of technique which has deterred many investigators from using glycaemia rather than glycosuria, as the criterion of change in the organism. Too often this has detracted greatly from the value of the research. Others have recognized the more fundamental bearing of variations in the

sugar of the blood but apparently without recognizing the great delicacy of the mechanism with which they were working. The result is that in many cases well-conceived experiments are largely vitiated by inadequate or improper controls. Hirsch and Reinbach and Rolly and Opperman[1] have recently called attention to the necessity of controlling, as far as possible, every factor in experiments of this nature.

The purpose of this paper is threefold: first, to determine, if possible, a set of conditions under which the amount of sugar in the blood of one laboratory animal, the cat, will be approximately constant. Upon such conditions, when once established, experimental conditions may be superimposed with reasonable assurance that differences from the constant are due to the new factors; secondly, to study the effects of some of the common conditions to which animals are subjected before being submitted to the experimental procedures in order that those which modify the concentration of sugar in the blood may be determined; and, lastly, to study, for the same reason, a few of the experimental procedures frequently used in experiments involving the estimation of the sugar in the blood. Abundant evidence from the literature, as well as my own work, shows that the most painstaking attention to all details is demanded if trustworthy results are to be obtained.

Although, as will be seen from the above, the experiments to be reported were not primarily planned to throw light upon the problems of the mobilization and use of sugar by the organism, it is thought that some of the results found may have a deep physiological significance. An extended discussion of the theoretical or possible significance of my results would, however, be out of place at this time. The attempt is made to discover and catalogue a few of those disturbing factors which are constantly entering into our experiments, unbidden and frequently without our knowledge, and which lead us to false conclusions. Many, perhaps most, of the factors studied by me have been previously investigated for other animals and indeed some of them for the cat. It was never-

[1] References to the literature cited will be found in Section VII, arranged alphabetically according to authors. Where more than one article is cited from one author the particular articles to which reference is made is indicated by the small numbers.

theless thought desirable to correlate the results for a single animal and by a single uniform method. In part because there has been comparatively little work done upon the cat, and in part for the reasons given below, this animal was selected for the research.

Long ago Boehm and Hoffman called attention to some of the advantages of cats for laboratory work. They mentioned especially their cleanly habits, their uniform size and the fact that they had found them to be more uniformly healthy than other common animals available for estimation of the sugar of the blood. There are, however, other and perhaps more fundamental advantages. A large amount of work has been done on excised muscles — a type of experiment for which the cat seems to be particularly adapted. Notes published by Lee and by Lee and Harrold show that some of this is directly related to the use of sugar by the organism. Again, some authors, as Macleod and Pearce, have sought to avoid, by decerebration, the extended use of drugs in experiments where prolonged anesthesia is necessary. The same results may be obtained in a more physiological manner, and with less hemorrhage, by cerebral anaemia. Leonard Hill and Stewart with his co-workers have shown that the dog, because of peculiarities of the blood-supply to the brain, is not so well adapted for this procedure as is the cat. Pike has confirmed Porter's statements that cats are better adapted for experiments involving vasomotor responses than are dogs.

II. — Method of Analysis

The preparation of the animal and method of obtaining the blood will be discussed later. Only the chemical processes involved will be described here. It is not possible to estimate the sugar by any known method in the presence of protein. Many reagents have been used and many methods proposed for the removal of the protein from blood preparatory to the determination of sugar. In 1908 Michaelis and Rona [1] proposed the use of colloidal iron hydroxide for this purpose. Their method has been well received and is widely used at present. Recently, however, Lesser reports that it is not satisfactory, in the form proposed by the authors for the blood of either frogs or turtles. In the

limited use that I have made of the method I have found it fairly satisfactory but have preferred the phosphotungstic acid method described below. A method which requires but a small amount of blood for the analysis possesses many obvious advantages. Because of this the method recently described by Lewis and Benedict or the micro-chemical methods of Bang or of Michaelis will prove of great value to both the clinician and the experimentalist, provided that they give the same satisfactory results in other hands that have been reported for them by their authors. Dehn and Hartman are now publishing a series of researches in which they are developing a method for the use of picric acid as the oxidizing agent in sugar determinations. Because of the greater delicacy claimed for it the picric acid method may supplant the use of copper for this purpose. The method which I have used is very similar to the one used by Pfeffer for the removal of proteins from bacterial cultures prior to the determination of sugar. Reid and, more recently, Oppler have described methods for removing the protein from blood by this reagent.

In my own method the blood was drawn directly from the blood-vessels into a beaker weighed with sufficient 1 per cent ammonium oxalate to make the final concentration of oxalate in the blood about 0.25 per cent. The beaker was constantly shaken while the blood was being drawn. The second weighing was made at once. Any blood on the sides of the beaker was then washed down with distilled water and about 300 cc. of water added. This was done for the double purpose of preventing glycolysis, as suggested by Rona and Döblin, and of breaking down the corpuscles so that any sugar contained within them might be freed. This was suggested by the work of A. Loeb, Rona and Michaelis,[2] Rona and Takahashi and others. As soon as the laking appeared complete the solution was washed into a 500 cc. volumetric flask, which was then filled to the mark with distilled water. It was then divided into two equal portions with the aid of a 250 cc. flask, and each portion was washed into a precipitation jar. About 1.2 cc. of a freshly prepared 10 per cent solution of phosphotungstic acid was then added for each gram of blood taken. This addition was made slowly from a dropping funnel while the mixture was being stirred with a mechanical stirrer. About twenty minutes was allowed for this precipita-

tion, but, thanks to the dropping funnels and the stirrers, did not consume much time on the part of the operator. The result was a brown or chocolate colored precipitate, from which a limpid filtrate rapidly separated, that gave none of the common protein reactions. After the precipitation was complete, each portion was washed into a 500 cc. flask, which was then filled to the mark, and filtered through an ordinary filter without suction. An aliquot part of about 350 cc. was taken for analysis.

The phosphotungstic acid was removed by the addition of 25 cc. of a saturated solution of barium hydroxide. After this addition, the mixture was allowed to stand for a time at room temperature. It must not be heated at this point, nor should it be allowed to stand much longer than is necessary to complete the reaction. The completion of the reaction was determined by the addition of a few drops of the barium solution to a few cubic centimeters of the clear supernatant fluid. When the reaction was complete it was again filtered and the precipitate was well washed with water. This filtrate was rendered just acid to litmus with sulphuric acid to precipitate the excess of barium, and the barium sulphate was removed by filtration. The final filtrate was evaporated to about 50 cc. in a Jena evaporating dish, and the sugar was estimated by the "Uniform method for sugar analysis" described by Munsen and Walker. Calculations were made from the tables given in Bulletin 107, Edition of 1912, of the Bureau of Chemistry, United States Department of Agriculture. The Bulletin, in addition to the table, contains a brief description of the method.

The accurate control of the estimation of sugar in blood or other solutions containing protein is very difficult. The present method was controlled as follows. A sample of blood was prepared as described above, except that a known quantity of glucose was added to one of the two portions just before the precipitation was begun. From this time on the estimation was completed in the usual manner. Evidently the amount of sugar recovered from the portion to which the addition was made, less the amount added, should be equal to that recovered from the other portion. Reference to Table 1 will show that this was the case within the limits of error permissible for work of this type. This method presupposes that the added sugar exists in the blood in the same condition as

TABLE 1

To show Degree of Recovery of Sugar. The Blood in Experiment 6 was Divided into Four Portions: *a*, *b*, *c* and *d*. Sugar was Added to Portions *c* and *d* as Indicated. In Experiment 17 the Blood was Divided into Three Portions and Sugar Added only to *c*. Experiment 20 was Carried out just as Described in the Text.

	Experiment No. 6				Experiment No. 17			Experiment No. 20	
	a	b	c	d	a	b	c	a	b
			Glucose added	Glucose added			Glucose added		Glucose added
Gm. blood in sample	47.51	47.51	47.51	47.51	49.41	49.41	49.41	50.67	50.67
Total glucose recovered ...	0.0314	0.0328	0.0501	0.0497	0.0334	0.0339	0.0514	0.0358	0.0538
Less glucose added........	—	—	0.0178	0.0178	—	—	0.0195	—	0.0195
Blood sugar recovered	0.0314	0.0328	0.0323	0.0319	0.0334	0.0339	0.0319	0.0358	0.0343
Blood sugar gm. per cent ..	0.0661	0.0690	0.0680	0.0671	0.0676	0.0686	0.0646	0.0705	0.0677
Av. of similar samples	av. a + b	0.0676	av. b + c	0.0676	av. a + b	0.0681	0.0646	—	—

that naturally present — a presumption which is by no means proved. For this reason, even though the results are satisfactory, one cannot be sure that all of the sugar has been recovered.

The degree to which results obtained by any one method are consistent, one with another, gives another means of judging of the accuracy of the method. The reader will have to be the judge of the way in which the present method responds to this test after having studied the tables submitted — especially Table 5.

Rona and Michaelis [1] have compared different methods for the removal of protein from blood and have found a variation in the amount of sugar recovered after the use of the several methods. This, they believe, is because the glucose does not all exist in the blood in simple solution. There is no present need for postulating the exact condition of the sugar, since any aggregation of the carbohydrate molecules or any combination of them with either protein or lipoid might easily interfere with the complete recovery of the dextrose by any of the methods available.

Some authors, notably Arthus, and Rosenfeld and Asher, have sought to show, by dialysis, that the sugar exists in the blood in simple solution. Consideration of the law of mass action, however, reveals the limitations of this method of attack. The equilibrium is at once destroyed by the removal of any portion of the sugar which may be in true solution. The disturbed condition will bring about a continuous dissolution of any loose combinations present so long as the removal occurs. In this way it is conceivable that a great deal of sugar may be removed by dialysis which did not originally exist in free solution.

From these considerations it follows that before the work of different authors or the results obtained by different methods may be compared, a factor of comparison must be established. That is, the amount of sugar recoverable from the same blood by each of the methods, under the same conditions, must be found, and the resulting ratios considered in making comparisons.

If it is true that the form in which the sugar is present in the blood influences the amount recoverable by the different methods, it follows that the amount recoverable by one and the same method may be expected to vary with the variation of the condition of the sugar or of any of its combinations which occurs as a result of

experimental procedures. Thus, the difficulty of interpretation of results all of which are obtained by the same method is also increased. We have at present no means of knowing that the sugar exists in the blood in the same state under different experimental conditions to which the animal has been exposed. Thus a rise or fall in recoverable sugar following any experimental change to which the animal may have been subjected, may be due to a change in the condition of the sugar in the blood with no variation in the absolute amount.

These possibilities of misinterpretation must be kept in mind in studying the following results.

III. — The Effect of Some of the Preliminary Conditions upon the Concentration of Sugar in the Blood

Under this head will come only those factors which, apart from the actual experimental conditions, interfere with the concentration of sugar.

The changes in environment undergone by an animal on entering the laboratory cannot be presumed to be without influence upon the point in question. Hence, if uniform results are to be expected, sufficient time must be allowed for all of the animals to establish themselves in equilibrium with their new surroundings. Of the many factors that might play a part in bringing about variations, two seemed especially liable to do this. These were, first, the changes in the character of the diet and feeding habits, and, secondly, the mental excitement incident to the new conditions. Time must be allowed the animals to establish themselves upon their new diet and to become accustomed to their new environment. Of the two, very probably the latter is the more productive of variations. A week seemed none too long a time to allow to the animals for this purpose, and hence was taken as the minimum limit in the usual routine. The few animals which were killed after a shorter period in the laboratory will be specially mentioned in the tables.

In Table 2 it is shown that the physical condition may be a disturbing factor. Here it is seen that the concentration of sugar may be high, as in numbers 58, 74 and 106, or be in essential

TABLE 2

To Show that the Concentration of Sugar in the Blood May be Disturbed by the Abnormal Physical Condition of the Animal

No. of Exp.	Sex	Body wt. k.	Blood drawn gm.	Blood per k. body wt. gm.	Gm. % glucose recovered	Var. from stand. mean—Table 5		Values calculated to 30 gm. blood per k. body wt.		Remarks
						Absolute variations	In % of stand. mean	Calculated concentration	% of var. from stand. mean	
31	—	—	126.88	—	0.075	+ 0.006	9	—	—	Abscess in jaw
58	M	2.99	107.35	35.90	0.107	+ 0.038	55	0.111	+ 61	Severe respiratory infection
60	M	2.41	75.40	31.28	0.062	− 0.007	10	0.063	− 9	Late recovery from respiratory inf.
61	M	2.92	72.91	24.93	0.068	− 0.001	1	0.064	− 7	Respiratory inf. early stages
74	M	2.50	59.43	23.77	0.086	+ 0.017	25	0.082	+ 19	Emaciated, cause unknown
95	M	2.35	79.69	33.91	0.047	− 0.022	32	0.050	− 28	Emaciated, unkempt, long standing respiratory inf.
106	M	3.12	88.80	28.46	0.101	+ 0.032	46	0.100	+ 45	Localized abscess on head

harmony with that of normal animals as shown by numbers 60 and 61; again in one case, number 95, which had apparently been running for a long time, the concentration was low. From this it is evident that one of the conditions for concordant results is the rigid exclusion of all animals which are not, so far as can be determined, in good health. While, as was shown above, a certain minimal stay in the laboratory should be allowed all animals before the sample is taken, too long a preparatory period is not desirable. The animals in general do not do as well in confinement as when free, and become especially liable to infection. For this reason, too, in experiments of long duration great care must be taken to protect the animal from all forms of infection and other influences which, aside from the purely experimental conditions, might lead to a changed physical condition.

The length of the period intervening between the last feeding and the collection of the blood may be an important factor. Bang and others have fed animals varying amounts of different carbohydrates in solution, and have followed the resulting changes in the concentration of sugar in the blood. Böe agrees with Bang that the hyperglycaemia induced in rabbits by this means has disappeared by the end of the third hour. Fischer and Wishart report a return to normal, in dogs which have ingested fifty grams of glucose in solution, by the end of the second hour. There is no doubt but that such experiments are of great value in determining the changes in glycaemia which take place under the conditions of the experiment. However, conclusions as to the conditions following an ordinary meal must be drawn with caution, since the time relations following the ingestion of protein and fat, or of these with starch, are not necessarily the same. This is true not only because of the different quantities of carbohydrate taken into the body under the different conditions, but also because of the difference in rates of absorption dependent upon the necessity for digestion in the usual meal and the interference arising from the other elements of the meal. In any case, it was thought best to allow sufficient time for any passing disturbance to disappear. With two exceptions the animals were allowed to live from sixteen to twenty-four hours after the last meal before the sample of blood was taken. In a number of cases the alimentary canals were

examined and found empty as far as the ileocoecal valve. Each of the two exceptions noted above were killed three hours after a meal, one of meat, the other of bread and meat. The one which had received meat alone, number 77, yielded 0.066 per cent sugar, which is, as will be seen by comparing with Table 5, in approximate agreement with the standard. The other cat, number 76, yielded a concentration of 0.086 per cent. This should, of course, be compared with the results shown in Table 4. When this is done it is seen that it is well within the limits of variation. Hence no significance can be attached to the variation in a single experiment from the average — 0.078 per cent — which is found for the corresponding series.

There seems to be some difference of opinion with regard to the effect of the character of the diet. Seelig finds less disturbance of the concentration of sugar in the blood of dogs given ether when the diet has consisted of bread for several days than when it has consisted largely of meat. As this point is of so much importance to the experimentalist, some attention was given to it. A diet consisting only of bread was found to be impractical for cats, so that they were given stale bread and cooked beef hearts, approximately pound for pound, together with the water in which the hearts were cooked. Even on this diet the animals did not do so well and were more subject to respiratory infection than those receiving the diet to be described later. It was not usually possible to keep them in a satisfactory condition on this diet for a longer time than two weeks. Aside from this, or perhaps because of it, a constancy of results for the quantity of sugar in the blood could not be obtained which approached that with the other diet. The results obtained are summarized in Table 4. It will be noticed that the variation between the extremes — 0.056 per cent and 0.104 per cent — is equal to 86 per cent of the smaller number and that 83 per cent vary from the average of the series by more than 10 per cent. Evidently this is not a satisfactory diet where a constant concentration of sugar is the end sought.

The other diet was cooked beef hearts with the bread omitted. This was found to be more satisfactory. While, as seen from Table 5, the extreme variation is between 0.096 per cent and 0.056 per cent and is thus almost as great as the variation of the previous

series, only 25 per cent of the animals vary from the mean by more than 10 per cent. Cf. Table 9.

Why the average for the animals allowed carbohydrate food in addition to the meat should be higher than that for those given meat alone, is a question difficult of answer. According to the ideas generally held, the character of the food is immaterial beyond the first few hours after the meal. Further, if the difference is due directly to the differences of diet, one would not look for the extreme variations which were found. There are other possibilities, however. Reference to Table 2 shows that animals with some types of infection seem to have a relatively higher content of sugar than the standard animals. As above noted, animals fed on the bread and meat diet are more prone to infection, and it is possible that in some cases incipient disease was overlooked. Again these animals were more restless and quarrelsome than those on the meat diet, and this would tend toward higher results. Rose thinks that carbohydrate feeding does not materially increase the amount of sugar in the blood of rabbits. It is, though, well to note in this connection that the rabbit is a herbivore, and as such may have better provision for handling carbohydrates than the cat, which is by nature a strict carnivore. One experiment reported by Rolly and Opperman [5] indicates that it is immaterial whether the protein given to the dog is derived from animal or vegetable sources. Jacobsen's [2] results are also of interest here.

It has long been known that the more intense emotions are a frequent cause of glycosuria. This was early spoken of by Rayer and somewhat later by Frerichs. Recently Cannon and some of his co-workers have laid especial stress upon this form of glycosuria; and have shown that for cats at least it is of purely psychological origin. Pavy [1] speaks of the necessity for "tranquillity" on the part of the animal while the sample is being drawn, and Eckhard emphasizes the fact that rabbits must not be tied in the holder for work involving glycosuria. Naunym very early reported an increased amount of sugar in the blood of animals which had been bound. Among the later writers Jacobsen,[1] Hirsch and Reinbach and Loewy and Rosenberg [1] have discussed in detail many of the difficulties in the way of the use of rabbits for experiments of this type. Presumably this difficulty lies, in large part at

least, in the nervous disposition of these animals and their proneness to excitement. Rolly and Opperman[3,5] discard them as entirely unsuited for such work. Seelig finds no glycosuria in one dog which had been bound for two and one-half hours. Rolly and Opperman[3] also think that dogs may be safely used for such experiments, while Loewy and Rosenberg on the other hand find the concentration of sugar in the blood of both dogs and of rabbits increased by sensory stimulation, though, it is true, the increase in the dogs was not so marked.

Boehm and Hoffmann first demonstrated glycosuria in cats as a result of binding them on a holder and so called it "Fesselung Diabetes." This result has been interpreted as being due to various factors, as mental excitement, loss of heat, and muscular exertion. It has been shown in Cannon's laboratory that the first factor alone is sufficient. He therefore suggests the term "emotional glycosuria." The general fact that hyperglycaemia and frequently glycosuria follow excitement in all laboratory animals, with the possible exception of the dog, and in man is widely accepted. The only reason for adding to the already extended literature of the subject is to find to what extent the handling of an animal which is necessary for obtaining a sample of blood or in preparing it for an experiment may disturb the standard conditions. In my experiments, all animals in which excitement was evident were discarded, except as noted in the tables. A few in which excitement was evident were killed, and the results proved the necessity of Pavy's rule of complete tranquillity if consistent findings are desired. Two animals, numbers 108 and 110, were held, as if given ether by a cone, though ether was not actually given in either case. A third was placed in a bell jar for about the length of time that would have been required for etherization, had ether been given. Others were subjected to other conditions which are apt to occur in the laboratory and which produced slight excitement, as indicated by crying or otherwise. The results, with a brief description of the conditions in each case, are given in Table 3. It will be seen that in every case there is a noticeable rise in the amount of sugar contained in the blood. These results show that the animal must be, as Pavy says, tranquil, not alone at the time that the sample is drawn, but for some time before. From

TABLE 3

To Show that the Ordinary Handling to which Animals are Subjected in the Course of an Experiment may Cause a High Concentration of Sugar in the Blood.

No. of Exp.	Sex	Body wt. k.	Blood drawn gm.	Blood per k. body wt. gm.	Gm. % glucose recovered	Var. from stand. mean-Table 5		Gm. % calculated to 30 gm. blood per k. body wt.		Remarks
						Absolute	% of mean	Concentration	Variation in % of stand. mean	
51	F	2.60	75.57	29.06	0.102	+ 0.033	48	0.101	46	Nervous in laboratory before killing
52	M	3.50	107.65	30.76	0.078	+ 0.009	13	0.078	13	In bag $1\frac{3}{4}$ hrs. before killing, quiet
64	M	2.11	66.03	31.29	0.169	+ 0.100	145	0.170	146	Excited when brought to laboratory
71	M	3.31	61.36	18.54	0.098	+ 0.029	42	0.091	32	In bell jar 3 min. just before killing
90	M	3.45	105.63	30.62	0.133	+ 0.064	93	0.133	93	Slight excitement before killing
92	F	2.10	61.90	29.48	0.149	+ 0.080	116	0.149	116	Excited by confinement in apparatus
108	F	2.19	45.86	20.94	0.122	+ 0.053	77	0.116	68	As 64: also held rigidly 3 min. before killing
110	F	2.20	56.86	25.85	0.086	+ 0.017	25	0.084	22	Held as 108 for 8 min., previously quiet

what has been said, it will be seen that those experiments in the past in which the blood has been drawn from an artery or vein without anesthetics have a very doubtful value, since it is hardly probable that an animal will undergo such an operation and remain in perfect tranquillity. The necessary restraint is of itself sufficient to influence the results as indicated by animals number 108 and 110. Of the disturbing effect of anesthetics more will be said later. There remains then only the possibility of rapidly killing the animal with the least possible excitement and the rapid withdrawal of the sample of blood after death. This was long ago appreciated by Pavy, who killed his animals by pithing with a Bernard needle and then collected the blood from the heart or from the thoracic cavity after severing the large blood vessels. Sudden decapitation and collection of the blood from the severed neck vessels seemed to offer some advantages over Pavy's method, and was used throughout the present work. The interval during which the animal was held before decapitation seldom exceeded three seconds, while about fifteen to twenty seconds more were required for the collection of the blood. As a further precaution against excitement, an attendant, from whom the animals were accustomed to receive food, brought them to the laboratory and assisted throughout the preparation of the animal and the collection of the blood.

That the amount of blood drawn relative to the total amount in the body may affect the concentration of sugar in the sample seems to have been overlooked by previous investigators. In studying this relationship a number of standard experiments were tabulated in the order of the increasing amounts of blood drawn, when this was expressed in grams per kilo of body weight. It was then found that the respective concentrations of sugar were arranged in the reverse order: that is, the more blood drawn per kilo of body weight, the lower is its concentration of sugar. This is wholly independent of the actual amount of blood drawn, as is shown below. The phenomenon is somewhat surprising since we know that hemorrhage under certain conditions causes hyperglycaemia. The relationship of which we are speaking, however, is not to be confused with the so-called "hemorrhage hyperglycaemia" as that term is commonly used.

In the curve shown in figure 1 the data are derived from Table 5. Unfortunately the body weights of the animals used in the earlier experiments were not recorded, hence these experiments are not available for our present use. The amounts of blood drawn

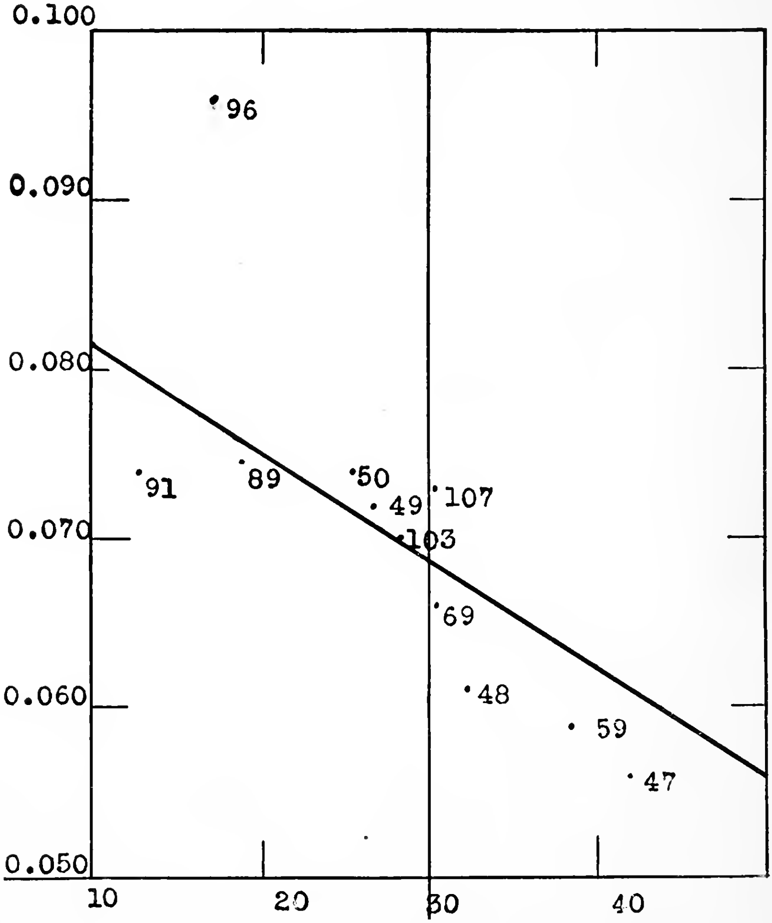

FIGURE 1

per kilo of body weight are plotted on the x-axis. The concentrations of sugar in the blood, expressed in grams per cent, were plotted on the y-axis. Provisionally the line represented by the equation $x/a + y/b = 1$ is taken as representing the relation found. In the equation x and y are variables; x represents the amounts

of blood drawn per kilo of body weight, and y the concentrations of sugar in the blood. a and b are constants whose values have not been exactly determined, but which approximate 133 and 0.084 respectively.

If this relationship between the amount of blood drawn and its concentration of sugar is constant from one animal to another, and if the above formula is its true expression, y is a constant in the equation $y = b\,(x' - x)\,/\,a + y'$; where a and b have the values assigned to them above. x' and y' represent respectively the amount of blood drawn per kilo of body weight and its concentration of sugar in any particular experiment. x may have any arbitrary value. Under these circumstances y represents the concentration which would have been found had x grams of blood per kilo of body weight been drawn. This calculation has been made for a number of the experiments, x being taken as equal to 30. The results, when recorded in the tables, are found in the column headed values calculated for 30 grams per kilo body weight. Since there have not been enough estimations made to warrant the assignment of exact values to a and b, the optimum position of the curve was ascertained by trial and the values were found mechanically after having plotted the results on coordinate paper as for figure 1. The results for the standard animals, calculated in the manner just described, are given in Table 5, column 10. A comparison of columns 7 and 10 of this table shows, first, that the mean for the series has not been modified; secondly, that the difference between the highest and the lowest results for the eleven animals compared is greatly reduced. Again the number of individuals which vary from the mean by more than 10 per cent is reduced from 4 to 1, or from 36 per cent to 9 per cent of the whole number of animals compared. Thus it will be seen that in general the calculated values approach still closer to a constant than do those derived directly from the analysis.

In order to test this relationship still further the blood was drawn from four animals in a series of samples in each of which the sugar was determined. The results obtained confirm those obtained by the former method and are given in Table 6. A study of this table reveals that in every case, with the exception of the third sample in experiment 95, the concentration of sugar in any sample

in the series is lower than for any sample in the same series previously drawn. These results do, however, indicate that the curve representing the relation is not a straight line, but that it falls more rapidly at first than it does later.

It was suggested that this relationship is due only to an error in analytical technique and so is of no physiological significance. For instance, it might be that a greater percentage of the sugar is recovered when only small amounts of blood are used. While it is true that such an error might lead to somewhat similar results, the

TABLE 4

The Effect of a Diet of Bread and Meat upon the Concentration of Sugar in the Blood of Cats

No. of Exp.	Sex	Days on diet	Body wt. k.	Blood drawn gm.	Blood per k. body wt. gm.	Gm. % glucose recovered	Var. from standard mean-table 5		Values cal. for 30 gm. blood per k. body wt.	
							Absolute	% of mean	Concentration gm. %	Var. % of standard
45	F	24	2.50	93.29	37.12	0.100	+ 0.031	45	0.105	+ 52
46	M	12	2.94	101.42	34.50	0.070	+ 0.001	1	0.073	+ 6
53	M	24	2.98	83.62	28.06	0.104	+ 0.035	51	0.102	+ 48
54	F	24	2.75	79.55	28.93	0.060	− 0.009	13	0.059	− 14
55	M	24	2.50	72.66	29.06	0.056	− 0.013	19	0.056	− 19
56	M	18	2.94	65.22	22.18	0.080	+ 0.011	16	0.075	+ 9
Mean						0.078	+ 0.009	13	0.078	+ 13

criticism cannot be valid for three reasons. First, it has been found that when the sugar in unequal samples of the same blood was determined, slightly smaller concentrations were found in the smaller samples. This was probably due to some slight negative error which has a tendency to be constant. This error would be multiplied by a larger factor when the absolute amounts recovered were computed to percentages and so lead to the smaller concentrations found in the smaller samples of blood. Thus it will be seen that in so far as this error would have any tendency, it would be to

hide the relationship found rather than to simulate it. Secondly, the effect is the same whether the variation in the amount of blood per kilo of body weight is brought about by drawing different amounts of blood from animals of approximately the same weight or by drawing the same amounts of blood from animals of different weights. Compare experiments 59 with 91, and 69 with 89 in Table 5.

TABLE 5

THE CONCENTRATION OF SUGAR IN CAT'S BLOOD UNDER THE CONDITIONS WHICH WERE SELECTED AS STANDARD

No. of Exp.	Sex	Days on diet	Body wt. k.	Blood drawn gm.	Blood per k. body wt. gm.	Gm. % glucose re-covered	Var. from standard mean, table 5		Values cal. for 30 gm. blood per k. body wt.	
							Absolute	% of mean	Concen-tration gm. %	Var. % of stan-dard
12	M	1	—	97.86	—	0.062	– 0.007	10	—	—
13	M	1	—	94.36	—	0.076	+ 0.007	10	—	—
15	—	1	—	129.73	—	0.063	– 0.006	9	—	—
16	—	1	—	60.94	—	0.063	– 0.006	9	—	—
23	—	5	—	116.33	—	0.070	+ 0.001	1	—	—
47	F	9	2.00	84.07	42.04	0.056	– 0.013	19	0.064	– 7
48	F	14	2.66	86.10	32.37	0.061	– 0.008	12	0.063	– 9
49	F	16	2.46	66.28	26.94	0.072	+ 0.003	4	0.070	+ 1
50	M	16	4.96	125.31	25.26	0.074	+ 0.005	7	0.071	+ 3
59	M	14	3.16	122.95	38.91	0.059	– 0.010	15	0.064	– 7
68	F	18	3.25	55.80	17.17	0.096	+ 0.027	39	0.088	+ 28
69	F	16	2.19	66.77	30.49	0.066	– 0.003	4	0.066	– 4
89	F	6	3.65	65.84	18.04	0.075	+ 0.006	9	0.068	– 1
91	M	25	3.05	39.56	12.97	0.074	+ 0.005	7	0.062	+ 10
103	M	8	2.23	62.61	28.08	0.070	+ 0.001	1	0.069	± 0
107	F	10	2.25	68.35	30.38	0.073	+ 0.004	6	0.073	+ 6
Mean	—	—	2.93	83.93	27.38	0.069	—	—	0.069	—

Thirdly, if the figures given in Table 6 are compared, it will be seen that the progressive decrease in concentration is entirely independent of the absolute amount of blood drawn.

No experiments have yet been made to determine the physiological significance of this decrease in the concentration of sugar when a larger proportion of the total blood is drawn at one time. The most plausible explanation which occurs to me is that it is due to the leaching of the tissue fluids into the blood vessels which occurs during severe hemorrhage. Evidence as to whether such a leaching does occur could be obtained by making simultaneous estimations of the sugar and the hemoglobin in the blood. Variations of the viscosity of the blood might also be used to throw light upon this question. Professor Burton-Opitz has found that blood drawn 25 to 30 minutes after a severe hemorrhage has a lower viscosity than that drawn before the hemorrhage. More than this, he assures me[1] that from his experience he would expect that the last of a large amount of blood drawn at one time would have a noticeably lower viscosity than would the first portions. This harmonizes directly with the theory advanced above.

Increased concentrations caused by the usual methods of withdrawal of blood have without doubt been the reason why the effect of the relative amount of hemorrhage upon the concentration of sugar in the blood has been so long overlooked. Factors are introduced by these methods which cause a greater or less discharge of the stores of glycogen, and so any small diminution in the concentrations of sugar in the blood is hidden. Pavy's method of collection should give the same results as mine, provided all the blood which has flowed from the vessels up to the time of collection is analyzed. However, I have been unable to find all of the necessary data in any of his tables. Schenck reports a very small difference between the first and second of two consecutive samples. In two experiments, the concentration of sugar in the second sample was less than in the first, and in one experiment, greater. Anderson, in two different experiments, finds almost the same concentration of sugar in each of three consecutive samples of blood. The later samples have, however, a slightly greater concentration of sugar than the earlier ones. Pavy[1] reports an increased amount of

[1] Personal communication.

sugar in the later samples of bullock's blood whether the animals were killed by the Jewish or by the pole-axe method. In none of the above were the samples so obtained that the discharge of stored glycogen would have been prevented.

Though probably sex of itself has no influence upon the concentration of sugar in the blood (cf. Bang), it is quite possible that the greater excitability of the male cats which Cannon [1] has found to exist may operate through the mechanism for emotional glycosuria to simulate such an influence. If this were true, it would be especially noticeable in experiments which involve much handling of the animal or its confinement in apparatus. While for animals kept under standard conditions I have found the mean for females slightly higher than that for males, the difference is small. Moreover, the nature of the individual variations, Tables 4 and 5, make one hesitate to attach any significance to this slight difference. It is quite possible that the results would have been otherwise for a series of animals confined in some apparatus, as a respiration chamber, for a period of time. The only evidence that I have on this is drawn from two animals which were confined in a small cage and exposed to a lowered temperature for a period of two hours. A small female cat, number 92, Table 3, resented the treatment and yielded a concentration of 0.149 per cent of sugar, while a large male, number 93, was apparently tranquil throughout the period, and yielded a result even lower than usual, 0.049 per cent. It seems to me that the stress should be laid upon the nature of the particular individual, rather than in blindly choosing animals of either sex. The sex of almost all the animals used is indicated in the tables. The results for some of the longer series are summarized in Table 7.

It is very doubtful whether there is any direct relationship between the body weight of the animal and the concentration of sugar in the blood, provided that the animals used are otherwise comparable. It must be borne in mind that variations in weight may be brought about by an abnormal physical condition, e.g., tuberculosis, and conversely these variations may be used as a means of detecting such abnormal conditions. A study of Tables 4 and 5 or of Table 8, in which the results bearing upon this point are summarized, reveals the fact that if the animals are divided

TABLE 6

THE RELATION BETWEEN THE AMOUNT OF BLOOD DRAWN PER KILO OF BODY WEIGHT AND ITS CONCENTRATION OF SUGAR

	Experiment 89 Body wt. 3.65 k.		Experiment 90 Body wt. 3.45 k.			Experiment 91 Body wt. 3.05 k.		Experiment 95 Body wt. 2.35 k.			
Order drawn	1st	2nd	1st	2nd	3rd	1st	2nd	1st	2nd	3rd	4th
Amount of blood gm.	44.89	20.95	54.2 (?)	29.29	22.04	25.47	14.09	17.55	13.93	10.43	37.79
Concentration of sugar	0.076	0.074	—	0.138	0.133	0.077	0.066	0.058	0.048	0.053	0.041
Remarks			1st sample spilled in tray, collected on weighed cotton and weighed. Not analyzed.								

TABLE 7

RELATION OF SEX TO AMOUNT OF SUGAR IN THE BLOOD

	Normal				Ether						Emotion	
	Meat		Bread and meat		Meat		Bread and meat		Street		Meat	
Sex	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female
No. of indiv	6	7	4	2	6	—	4	7	4	4	4	4
Gm. per cent glucose	0.069	0.071	0.077	0.080	0.152	—	0.192	0.166	0.129	0.102	0.120	0.115

into two series, those heavier than the mean and those lighter than the mean, the average concentration of sugar in the blood of the former is slightly higher than in the latter. This difference is, however, slight, and a study of the individual variations indicate that there is no direct relationship between body weight and the concentration of sugar in the blood.

TABLE 8

SHOWING THE INDEPENDENCE OF BODY WEIGHT AND THE CONCENTRATION OF SUGAR IN THE BLOOD OF HEALTHY CATS

	Meat fed Gm. % of sugar		Bread and meat Gm. % of sugar	
	Actual	Calculated to 30 gm.	Actual	Calculated to 30 gm.
Gm. per cent for heaviest animal in series	.C74	.071	.105	.102
Gm. per cent for lightest animal in series	.056	.064	{ .100 .056	{ .104 .056
Average body wt. for series k.	2.93	2.93	2.77	2.77
Wt. of animal with most sugar	3.25	3.25	2.98	2.50
Wt. of animal with least sugar	2.00	3.05	2.50	2.50
Gm. per cent sugar for those heavier than mean	.076	.C71	.085	.083
Gm. per cent sugar for those lighter than mean	.066	.068	.072	.073

IV. — THE CONTENT OF SUGAR IN THE BLOOD OF STANDARD ANIMALS

Before an interpretation of experimental work may be legitimately attempted, a standard must be fixed as a basis for comparison. It must, however, be clearly kept in mind that such a standard is no more normal than many other values which might be obtained. One ot the most striking characteristics of almost all of the published tables showing the concentration of sugar in the blood is not the constancy which we have been led to expect, but a variation within rather wide limits.

One of our criteria of life is the ability of the organism to respond to changes in the environment. That is, in any environment, the organism tends to reach a condition of equilibrium and is successful in life in so far as it is successful in maintaining itself in equilibrium with its constantly changing environment. This has long been recognized for external and physical conditions, so that one would hardly say, for example, that an animal was more normal standing than walking, or asleep than awake. But internal, including chemical, readjustments must occur which are just as normal as are the more obvious physical responses. Mathews has recently spoken of the general bearing of this class of adjustments. Cannon[2] has selected the concentration of sugar in the blood of animals undergoing emotional disturbance as a type of such readjustment. He holds, and with reason, that there may be as much "purpose" in this reflex as there is in the accompanying muscular response. Indeed, the increased amount of mobilized sugar may be necessary to make the more obvious muscular response possible.

It is then hopeless to think of finding any one value which will be closely approximated by all normal animals. However this may be, the more nearly we subject the animals to a standard set of conditions before the sample of blood is obtained, the more nearly we may expect to approach a constant value. With organisms so complex as are the mammals, absolute constancy of the preliminary conditions is manifestly impracticable, and so we can hardly expect an absolutely constant value for the concentration of sugar in the blood. Again, after having established a standard value for the concentration of sugar under some one set of conditions, modification of any one or more of the factors might be expected to give a new, but none the less normal, value. Thus the addition of bread to the diet might well give a different value than meat alone (compare Tables 4 and 5).

The method of preliminary treatment which in my hands has given the most constant results, together with some of the factors which may bring about variations, has already been described. The results are given in Table 5 (compare also the values given in Table 1). Results in the other tables are to be compared with those in Table 5 as a standard, since the experiments have been

made upon animals which might otherwise have been presumed to have given similar results.

These considerations, together with the relation between the condition of the sugar in the blood and the method of analysis, make it obvious that at present only relative values for the concentration of sugar in the blood are to be expected. In order that the results of a research may be comparable, a set of preliminary conditions, which have been shown to give an approximately constant concentration of sugar, must be selected as a standard. The exact nature of these conditions will, presumably, be determined, to some extent at least, by the nature of the particular research in hand. Before the results of different researches may be properly compared, they must be reduced to similar terms. This may be done by a factor of comparison similar to the one described on page 277, but which includes the preliminary conditions as well as the method of analysis.

There seem to have been but comparatively few determinations of the sugar in cat's blood. Boehm and Hoffmann made 26 observations on blood drawn from the carotid without anesthesia. Their results are, as one would expect, high, varying between 0.11 per cent and 0.31 per cent. They may well be considered examples of emotional hyperglycaemia and should be compared with my results given in Table 3, rather than with the standard results in Table 5. Rona and Takahashi report analyses of the blood from four cats. They too drew the blood from the carotid, but under light narcosis. The concentrations which they found are quite comparable in magnitude with those of Boehm and Hoffmann, varying between 0.154 per cent and 0.355 per cent. The high concentration here is, however, probably due to the anesthetic and should be compared with results shown in Tables 10–12 rather than with my standard results. Pavy [2] gives the results of six analyses of blood taken from the heart after pithing. The values vary between 0.068 per cent and 0.1026 per cent, with a mean of 0.088 per cent. Since the type of diet and general preliminary treatment are not given, one cannot tell to what extent his results are comparable with mine, or whether they should be compared with my standard or with the results for cats fed on bread and meat which are given in Table 4. The above results are summarized in Table 9.

TABLE 9

TABLE GIVING SUMMARY OF CONCENTRATIONS OF SUGAR IN CAT'S BLOOD FOUND BY DIFFERENT OBSERVERS

Observer	Manner of collection	No. of observations	Mean concentration in %	Highest concentration	Lowest concentration	Observations which vary from average by more than 10%	
						Absolute no.	% of whole no.
Boehm and Hoffmann .	From carotid no anesthesia	26	0.15	0.31	0.11	21 (?)	81
Rona and Takahashi .	From carotid light narcosis	4	0.282	0.355	0.154	3	75
Pavy.......	From heart after pithing	6	0.088	0.103	0.068	5	83
Scott.......	From neck vessels after decapitation	22	0.069	0.096	0.056	4	18

V. — THE RELATION OF A FEW OF THE ORDINARY EXPERIMENTAL PROCEDURES TO THE CONCENTRATION OF SUGAR IN THE BLOOD

From what has been said it will be seen that any experiment which involves the estimation of the sugar in the blood would be valueless if the animal is subjected to pain or other form of excitement during the course of the experiment or within a few hours previous to it. It will also be noted that this is quite apart from any humanitarian considerations. Any work therefore which would otherwise involve pain must be accompanied by an anesthetic. This at once brings up the question of the effect of the anesthetic itself.

That ether, occasionally at least, causes glycosuria in patients undergoing operations has been known almost from the beginning of its use as an anesthetic. Harley and Tiegel very early demonstrated glycosuria in animals to which ether had been given. Hawk maintains that it always occurs in dogs when ether is used as an anesthetic, and his statements have been confirmed by Seelig.

Underhill publishes figures showing an increase in the concentration of sugar in the blood of two dogs which had received ether.

The fact that the administration of ether is accompanied by hyperglycaemia does not of itself preclude its use in experiments of this character. If a set of conditions — of which ether is one — can be found that meets the requirements of a standard, it would seem that the use of ether would be legitimate. In the use of ether, difficulty is at once encountered in getting the animal under the influence of the drug without introducing other disturbing factors. In Table 3 it was shown that the rigid holding of the animal necessary in the use of the cone for this purpose is productive of a significant disturbance in the concentration of sugar in the blood. Likewise, in the single case tried, a similar result was obtained when the animal was confined under a bell jar. This particular animal, however, resented the confinement. It was found that by careful selection of individuals those could be found which so far as one could tell were not disturbed by the brief restraint necessary. The bell jar has the disadvantage of offering greater danger of partial asphyxiation than does the cone, and it has been abundantly shown that asphyxia of itself is sufficient to cause hyperglycaemia (cf. Bang). It was thought that with proper precautions any danger of asphyxia could be avoided and that aside from this there were fewer objections to the use of the bell jar. Consequently in all of my experiments, where ether or chloroform was given, the animal was put in a bell jar for the initial stages. The animals were removed from the jar as soon as muscular relaxation had occurred. When the anesthetic was to be given for a longer time, this was done by means of a cone. Asphyxia was avoided either by very rapid anesthetization in a jar of fairly large volume or by the admission of air below the jar when slower anesthetization was desired.

The results for animals prepared in the standard manner are shown in Table 10. It is evident that there is no approximation to a constant. And in addition to this the animals to which ether was given for 30 minutes have a distinctly higher concentration of sugar in their blood than those to which it was administered for only three minutes or less. This indicates a cumulative effect of the ether, which would still further confuse the results of the experiment.

TABLE 10

THE AMOUNT OF SUGAR IN THE BLOOD OF CATS PREPARED IN STANDARD MANNER + ETHER

No. of Exp.	Sex	No. of days on diet	Body wt. k.	Time in min. from beginning of etherization		Amt. of blood gm.	Concentration glucose gm. %	% var. from		Values calculated. for 30 gm. blood per k.		
				To musc. relax.	To death			Mean this series	Mean stand. series	Concentration	% of var. from mean of	
											This series	Stand. series
66	M	16	3.45	.92	1.00	79.38	0.113	+ 3	+ 64	0.108	+ 1	+ 57
73 [1]	M	12	3.29	2.67	3.00	97.20	0.132	+ 20	+ 91	0.132	+ 23	+ 91
74	M	12	2.50	2.17	2.17 +	59.43	0.086	– 22	+ 25	0.082	– 23	+ 19
Mean	—	—	—	—	—	—	0.110	—	+ 59	0.107	—	+ 55
78 [2]	M	14	3.91	1.50	30	89.45	0.133	– 32	+ 93	0.128	– 34	+ 86
81	M	14	2.10	3.67	30	71.31	0.302	+ 54	+ 338	0.305	+ 56	+ 342
86	M	9	2.80	2.75	30	84.43	0.153	– 22	+ 124	0.153	– 22	+ 124
Mean	—	—	—	—	—	—	0.196	—	+ 184	0.195	—	+ 183

[1] Slight struggling when brought to laboratory.
[2] Shivering last ten minutes.

Seelig reports that ether gives much less trouble in this way with dogs which have been fed on bread for some time than with those which have been on a meat diet. Macleod also has sought to avoid the disturbing effect of ether in the same way. The results which I have obtained with cats fed on the bread and meat diet described on page 281 are shown in Table 11. A comparison of these results with those given in the preceding table shows that while there is still so much variation that they would be unsatisfactory as a basis for experimental results, they are more uniform than those obtained with the meat diet. Also the cumulative effect of the ether is not so great.

A definite relation between the ease with which the equilibrium of the mobile carbohydrates of the body is disturbed and the type of diet given the animal would be of considerable theoretical interest. Such a difference must imply a difference either in the chemical form of the carbohydrate or in the tissues in which it is stored. This theoretical interest, together with the opportunity which might be offered the experimentalist of reducing the variations to a minimum, would warrant sufficient work to establish either the existence or non-existence of such a relation. This is especially true, since Seelig's results agree with those given above in indicating the hopeful outcome of such a research.

The results given in Table 12 were obtained from animals which were to be used by a class of medical students. These animals were killed by decapitation as usual, but without special preparation. The first eight were anesthetized by ether in a bell jar in the usual manner by the students. As soon as muscular relaxation had occurred, they were removed from the jar and decapitated at once. The last five were used for demonstration purposes and had been under ether for periods varying from an hour to three or four hours, during which time the operation indicated had been done. Since these animals were primarily used for other purposes, I was unable to record the full data. The results are, however, given in the hope that they will prove of some value, indicative as they are of the results which may be expected under ordinary laboratory conditions. Chloroform does not seem to offer any advantages over ether (Harley) and has the disadvantage of a greater toxicity. A few experiments of my own, likewise, give no indication of any

TABLE 11

THE CONCENTRATION OF SUGAR IN THE BLOOD OF CATS FED ON BREAD AND MEAT AND WHICH HAVE BEEN GIVEN ETHER

No. of Exp.	Sex	No. of days on diet	Body wt. k.	Time in min. from beginning of etherization		Amt. of blood gm.	Concentration glucose gm. %	% Var. from		Values calculated for 30 gm. blood per k.		
				To musc. relax.	To death			Mean this series	Mean stand. series 0.078	Concentration	% of var. from mean: This series	% of var. from mean: Stand. series 0.078
63	F	11	2.49	1.00	1 +	79.99	0.172	+ 15	+ 120	0.173	+ 16	+ 122
65	F	12	2.45	1.17	1.17 +	63.42	0.135	– 9	+ 73	0.132	– 11	+ 69
67	M	17	2.12	1.67	2.67	53.30	0.170	+ 14	+ 118	0.167	+ 12	+ 114
70	F	19	2.17	3.50	3.50 +	58.82	0.166	+ 11	+ 113	0.164	+ 10	+ 110
72	F	20	1.84	3.00	4.00	52.34	0.142	– 5	+ 82	0.141	– 5	+ 81
79	F	23	2.26	2.17	2.17 +	80.55	0.111	– 25	+ 42	0.115	– 23	+ 47
Mean	—	—	—	—	—	—	0.149	—	+ 91	0.149	—	+ 91
75	M	8	2.02	3.50	24	70.93	0.213	+ 3	+ 173	0.216	+ 6	+ 177
80	M	8	3.35	2.50	30	61.22	0.215	+ 4	+ 176	0.208	+ 2	+ 167
82	F	7	2.80	3.83	67	65.30	0.155	– 25	+ 99	0.150	– 26	+ 92
83	M	7	2.25	1.50	29	57.06	0.171	– 17	+ 119	0.168	– 18	+ 115
94	F	29	2.65	4.00	34	80.48	0.280	+ 35	+ 259	0.280	+ 37	+ 259
Mean	—	—	—	—	—	—	0.207	—	+ 165	0.204	—	+ 161

TABLE 12

EFFECT OF ETHER ON CONCENTRATION OF SUGAR IN BLOOD OF CATS WHICH HAVE RECEIVED NO ESPECIAL PREPARATION

No. of Exp.	Sex	Body wt. k.	Amt. of blood gm.	Concentration sugar gm. %	Per cent of variation from mean of			Remarks
					This series	Stand. series	Bread and meat series	
37	M	—	80.05	0.106	− 8	+ 54	+ 38	
38	F	—	75.25	0.104	− 10	+ 51	+ 33	
39	M	—	97.25	0.151	+ 31	+ 119	+ 94	
40	F	—	52.60	0.094	− 18	+ 36	+ 21	
41	M	—	80.60	0.123	+ 7	+ 78	+ 58	
42	M	—	63.25	0.133	+ 16	+ 93	+ 71	
43	F	—	49.45	0.126	+ 10	+ 83	+ 62	
44	F	—	62.05	0.084	− 27	+ 22	+ 8	
Mean	—	—	—	0.115	—	+ 67	+ 47	
25 [1]	—	—	91.25	0.129	− 40	+ 87	+ 65	Pleural puncture, treacheotomy
57 [1]	M	3.00	80.89	0.134	− 38	+ 94	+ 72	Decortication
62 [1]	M	3.25	55.66	0.239	+ 11	+ 246	+ 206	Decortication less ether than no. 57
84 [1]	M	2.80	79.92	0.298	+ 39	+ 332	+ 282	
85 [1]	M	2.75	72.80	0.274	+ 27	+ 300	+ 251	Respiration stopped under ether
Mean	—	—	—	0.215	—	+ 212	+ 176	

[1] These animals were used for class demonstration and were under ether for at least one hour and in addition were subjected to the operations indicated.

advantage to be derived from its use, since the results are essentially similar to those obtained by the use of ether (Table 13).

Because of its stimulating action on the cat, no one would think of making use of morphine in drawing blood from this particular animal. It is, however, of interest to note that Luzzatto finds glycosuria following the use of morphine in rabbits. This finding is confirmed by Araki, who also reports similar results for

TABLE 13

THE RELATION BETWEEN CHLOROFORM AND THE CONCENTRATION OF SUGAR IN THE BLOOD OF ANIMALS PREPARED IN THE STANDARD MANNER

No. of Exp.	Sex	Body wt. k.	Time in min. from application of chloroform to		Amt. of blood drawn gm.	Concentration sugar gm. %	% var. from mean		Remarks
			Musc. relaxation	Death			This series	Stand. series	
87	M	3.79	5.0	5.17	78.05	0.105	− 9	+ 52	Unusually quiet before and during anesthetization
88	M	3.50	2.0	2.67	67.94	0.098	− 15	+ 42	
97	F	2.75	1.5	1.57	62.96	0.142	+ 23	+ 105	
Mean	—	—	—	—	—	0.115	—	+ 67	

dogs, though he found no sugar in the urine of frogs after morphine. On the other hand, Hirsch and Reinbach think that morphine is without effect on the concentration of sugar in the blood of rabbits. Jacobsen found an undoubted increase in the amount of sugar in the blood of rabbits to which sufficient chloral had been given to produce narcosis.

Some investigators have collected blood from one of the large vessels under the local anesthesia produced by cocaine (Fisher and Wishart). For some types of experiment, such a method is particularly desirable, provided the equilibrium of the mobile sugar is not disturbed by the drug in such a manner that the proper allowances cannot be made. Araki found lactic acid in the urine of frogs and of rabbits after the injection of cocaine. One of the four rabbits injected also secreted sugar with the urine. In the present work four cats were injected beneath the skin of the back with large doses of cocain hydrochloride dissolved in N/8 sodium chloride solution. These animals were all killed in the early stages of the apparent reaction to the drug. (See Table 14 for details.) With one exception each of the concentrations of sugar found was well below the standard concentration. The mean concentration for the series is 86 per cent of the standard mean. Any attempt to explain this finding woulP be premature, since a longer series

TABLE 14

THE EFFECT OF COCAINE UPON THE CONCENTRATION OF SUGAR IN THE BLOOD OF CATS PREPARED IN THE STANDARD MANNER

No. of Exp.	Sex	Days on diet	Body wt. k.	Amt. cocaine injected gm.	Time between injection and death in min.	Amt. of blood drawn gm.	Concentration sugar in blood gm. %	% Var. from mean of		Remarks
								This series	Stand. series	
98	F	2	2.58	0.07	8	68.79	0.063	+ 7	– 9	Killed in 1st stages of stimulation by drug
100 [1]	M	3	2.85	0.05	10	87.40	0.055	– 7	– 20	No other excitement
101	F	6	2.37	0.07	6	57.38	0.048	– 19	– 30	As No. 98
102	M	8	2.68	0.07	6	77.85	0.070	+ 19	+ 1	Killed after symptoms of the drug were marked
Mean	—	—	—	—	—	—	0.059	—	– 14	

[1] Only slight symptoms of drug at time of death.

might yield results which would essentially modify the situation. Also too little is known at present of the other factors of metabolism during intoxication by cocaine to warrant such an attempt.

In following the progress of an experiment it is frequently desirable to determine the changes in the concentration of the sugar in the blood at frequent intervals. Unfortunately there is, however, a very serious objection to this procedure. Claude Bernard found that the concentration of sugar is increased by a previous hemorrhage, and his finding has been repeatedly confirmed. Recently fairly exhaustive studies have been made by several authors. Among others Anderson, Jacobsen, Rose and Schenck have studied this effect in rabbits. Anderson found an increased concentration of sugar five minutes after the hemorrhage, but did not determine whether it was present after a still shorter interval. The consensus of opinion is that the concentration reaches its maximum about thirty minutes after the hemorrhage and that it remains high from three to four hours.

Undoubtedly emotional disturbances have frequently contributed a large share to the so-called hemorrhage hyperglycaemia. However this may be, there is no doubt that quite apart from any disturbance due to emotion or to anesthetics, hemorrhage does introduce a modification for which proper controls must be made. Some authors have sought to avoid the introduction of the factor of hemorrhage by the use of a quantity of blood so small that it might be considered as negligible, but this so greatly increases the probable error from analytical technique that the method has a questionable value, at least for most methods of analysis. Furthermore the objection to repeated handling of the animal and the consequent excitement are not met by the change in analytical method, and demand exceptional skill on the part of the experimenter. The literature covering this subject is so ample and, taken as a whole, so conclusive that it was not thought necessary to add to it.

Changes in the concentration of the sugar in the blood may be used as a measure of the effect of a substance which has been injected into the animal. In such experiments it is usually presumed that the effect of the injection aside from the drug is nil. My own experiments are too few in number to allow of general conclusions.

But in harmony with the rest of my work they indicate the necessity of complete control of all factors in the experiment. Long ago Bock and Hoffmann showed that large amounts of salt solution caused glycosuria when injected intravenously. But while drugs are frequently dissolved in a solution of sodium chloride for injection, the effect of the salt solution is essentially different from that obtained by Bock and Hoffmann, since usually very much smaller amounts are injected. In my own experiments with cocaine certainly no factor was introduced which increased the concentration of sugar enough to conceal the results due to the cocaine alone, with the possible exception of one animal. (See Table 14.) Especial care was taken in making these injections of cocaine to avoid exciting the animal. The same care was exercised in an animal which was injected with 5 cc. of M/8 sodium chloride. This cat was killed five hours later, and the blood yielded a concentration of sugar of 0.0697 per cent, a result almost exactly the same as the standard. Another animal injected through an opening in a small box in which it was confined with the same amount of sodium chloride solution became much excited. This animal, after the lapse of a similar interval, yielded a concentration of 0.098 per cent — a much higher result than the standard.

Again, many experiments of this nature involve the confinement of the animal within some form of apparatus. The exact results obtained in animal calorimetry are ample evidence of the availability of this type of research. On the other hand, great care is necessary to avoid exciting the animal. This is illustrated in the two animals exposed to cold as described on page 291. While they were exposed to similar external conditions, one became very restless and yielded a concentration of 0.149 per cent of sugar; the other remained exceptionally quiet and yielded a concentration of only 0.049 per cent. The nature of this experiment, together with the results, would suggest the possibility that the unpleasant conditions involved constitute at least one of the factors leading to the mobilization of the sugar in Lusk's method of ridding the body of glycogen by shivering.

In another experiment four cats were confined in a respiratory chamber and subjected to a temperature of about 32° C. and a relative humidity of about 88 per cent. The results are shown in

Table 15. Animals were selected for these experiments which might be expected to remain quiet throughout the experiment. No. 112 was the only one which proved disappointing in this regard. The results show an exceptionally low average for the concentration of sugar, and this individual is the only one of the four which reached the level of the standard cats.

TABLE 15

THE EFFECT OF THE CONFINEMENT OF CATS IN A WARM, MOIST CHAMBER UPON THE CONCENTRATION OF SUGAR IN THE BLOOD

No. of Exp.	Sex	Body wt. k.	Amt. of blood drawn gm.	Hrs. in chamber	Temp. mean C.	Rel. humidity mean	Concentration sugar in blood gm. %	% of var. from stand. mean	Cal. for 30 gm. blood per k. body wt.	
									Concentration sugar gm. %	% var. from stand. average
111	F	3.65	83.38	6	30.6	.83	0.053	– 23	0.049	– 29
112 [1]	F	1.62	55.57	6	30.7	.89	0.064	– 7	0.067	– 3
113	F	3.78	69.87	6	33.1	.90	0.065	– 6	0.058	– 16
114	F	2.90	65.12	6	32.9	.90	0.059	– 14	0.054	– 22
Mean	—	—	—	—	31.8	.88	0.060	– 13	0.057	– 16

[1] Excited when removed from the chamber.

There seems then to be no reason for attributing changes in the concentration of the sugar in the blood following the injection of small amounts of salt, or the confinement of the animal in apparatus to these conditions of themselves. Excitement induced by these conditions may, however, give rise to high concentrations, even five hours after the time of irritation.

VI. — SUMMARY AND CONCLUSIONS

1. Glycaemia offers a more satisfactory indication of the condition of mobile sugar than does glycosuria; first, because either an increase or a decrease in the amount of sugar may be demonstrated, while normal urine can show only an increase; secondly,

because profound changes in glycaemia may occur in response to conditions which do not produce glycosuria; thirdly, the blood is in much more direct relation to the living cells than is the urine.

2. The concentration of sugar in the blood as estimated by different methods varies. This may be due, in part at least, to the form in which the sugar is present in the blood. It follows that results obtained by different methods of analysis cannot properly be compared until they have been reduced to common terms. Also the possibility is introduced of an apparent variation in concentration of sugar, even when the method of estimation is constant, which is due to a change in the form in which the sugar is present rather than to a change in the actual amount of sugar present.

3. If consistent results are to be expected, the animals must be uniformly healthy, and must be killed without pain or excitement. Sex or weight, apart from correlated conditions, are probably without special influence upon the concentration of sugar in the blood.

4. The normal concentration of sugar may very probably vary with the varying environment of the animal or with changes in its physical state. However, if the environment is uniform and if the animals are killed while in the same physiological condition, constant results should be expected. Practically such an ideal result is not possible, but has been approached with some success.

5. The concentration of sugar in the blood decreases as the amount of blood drawn per kilo of body weight of the animal increases. So far sufficient data have not been obtained to establish the mathematical expression for this relation.

6. When ether or chloroform was administered, the concentration of sugar was increased considerably and varied between rather wide limits, whether the diet consisted of meat alone, or of bread and meat, the latter diet giving somewhat smaller variations than the former. After either diet there was a greater concentration after the drug had been administered for thirty minutes than after it had been administered for three minutes or less.

7. The concentration of sugar in the blood after subcutaneous injection of cocaine is more constant than that found after inhalation of ether or chloroform and is lower than that found in animals similarly treated but to which cocaine has not been given.

8. It may be shown from the literature that hyperglycaemia

follows hemorrhage. From this it follows that caution must be exercised in drawing conclusions from experiments which involve the analysis of successive samples of blood.

9. The excitement which is apt to attend hypodermic injections or confinement in apparatus may lead to high results and consequently to false conclusions, With care, however, such effects may be avoided so that this type of experiment is permissible.

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VITA

I was born on August 18th, 1877, in Kinsman, Ohio, and graduated from the Kinsman High School with the class of 1897. In 1902 I received the degree of Bachelor of Science from Ohio Wesleyan University. Immediately upon graduation I entered the United States Coast and Geodetic Survey. While in this service the greater part of my time was spent in hydrographic work in Chesapeake Bay.

I began my duties as instructor in sciences in the Western High School, Bay City, Michigan, in September, 1903, and remained there until April, 1910, when I accepted a fellowship in the Department of Physiology of the University of Chicago. With the beginning of the following summer quarter my rank was raised to that of assistant. While in the University of Chicago a part of my time was taken up with instruction in laboratory courses and a part was given to study and research. In the spring of 1911 I was elected to Sigma Xi and at the close of the summer quarter received the degree of Master of Science. My thesis, which represented an experimental investigation of the influence of intravenous injections of an extract of the pancreas on experimental pancreatic diabetes, was published in the American Journal of Physiology, volume twenty-nine, 1912.

In the autumn of 1911 I entered upon my duties as assistant professor of physiology in the University of Kansas. At the close of one year I resigned this position to accept an instructorship in physiology in Columbia University. I have now been advanced to an associateship in Columbia for 1914–15.

(Signed) ERNEST LYMAN SCOTT

May 10, 1914.

Scott

The content of sugar in the blood.

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